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(19) (CA) **CANADIAN PATENT** (12)

(54) Chondroitin Sulfate/Sodium Hyaluronate Compositions

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The invention relates to compositions obtained by adding chondroitin sulfate to sodium hyaluronate in aqueous buffer solution, or sodium hyaluronate to 5 chondroitin sulfate in aqueous buffer solution.

10

This invention relates to compositions for protecting both human and animal endothelial and epithelial cells which are subject to exposure to trauma. More particularly, this invention concerns 15 compositions for protecting endothelial and epithelial cells in anticipation of surgical trauma using chondroitin sulfate/sodium hyaluronate compositions.

Since human corneal endothelial cells are 20 not known to reproduce, it is of vital importance to protect endothelia to prevent cell damage prior to subjection to anticipated trauma, such as surgery. Recent advances in ophthalmic surgery have increased 25 the need to protect corneal endothelial cells which may be subject to irreversible destruction during such surgery. Of particular significance is the need to protect corneal endothelial cells during intraocular lens (IOL) implantation, corneal transplantation and other intraocular surgical operations. Previous work 30 in this field has been directed to protecting corneas with both non-biological and biological polymers.

Macromolecules heretofore employed in the protection of corneas include chondroitin sulfate and



A
5 sodium hyaluronate. The use of a chondroitin sulfate solution for the protection of corneal surface tissue is described in a "CHONDRON" * product monogram, Kakan Pharmaceutical Company, Ltd., Tokyo, Japan, 1981. The use of sodium hyaluronate as an aid in ophthalmic surgery is described in a "HEALON" * product monogram, Pharmacia Laboratories, Piscataway, New Jersey, 1980.

10 Solutions containing chondroitin sulfate or sodium hyaluronate alone have not met with complete satisfaction due to inadequate corneal dome maintenance which in turn provides spatial separation of cornea endothelium and surgical instruments, etc., or inadequate corneal endothelial cell protection, respectively.

15 In view of the above, it would be advantageous to prepare a viscous composition containing chondroitin sulfate and sodium hyaluronate, without the use of any other active material which could irritate or damage corneal endothelial cells.

20 Viscosity is normally a function of molecular weight at constant solute concentration. It has now been discovered that chondroitin sulfate and sodium hyaluronate may be mixed in aqueous buffer solution in specified ratios to produce a composition having surprisingly high viscosity and offering protection to corneal surface cells during 25 intraocular lens implantation, corneal transplantation, and other intraocular surgical operations, which is superior to that obtained with either of the individual components.

30 Additionally, the chondroitin sulfate/sodium hyaluronate compositions of the present invention can be administered after trauma as an aid in healing. Surprisingly, it has been found that the chondroitin sulfate/sodium hyaluronate compositions of the present invention exhibit enhanced solution stability and improved physical properties by comparison with the individual 35 components of the composition. These compositions are

* Trade Marks

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useful for topical applications as well as for irrigation during surgery.

Thus according to the present invention there is provided a method to protect both human and animal cell layers and tissues subject to exposure to trauma which comprises administering a therapeutically effective amount of a viscous aqueous composition comprising a chondroitin sulfate compound selected from chondroitin sulfate, sodium chondroitin sulfate, potassium chondroitin sulfate, magnesium chondroitin sulfate and calcium chondroitin sulfate, and a hyaluronate selected from sodium hyaluronate, potassium hyaluronate, magnesium hyaluronate and calcium hyaluronate, to said cell layers and tissues prior to said exposure to said trauma, said aqueous composition comprising chondroitin sulfate and sodium hyaluronate in an aqueous buffer, and exhibitin, a viscosity which exceeds the individual viscosity of said chondroitin sulfate and sodium hyaluronate.

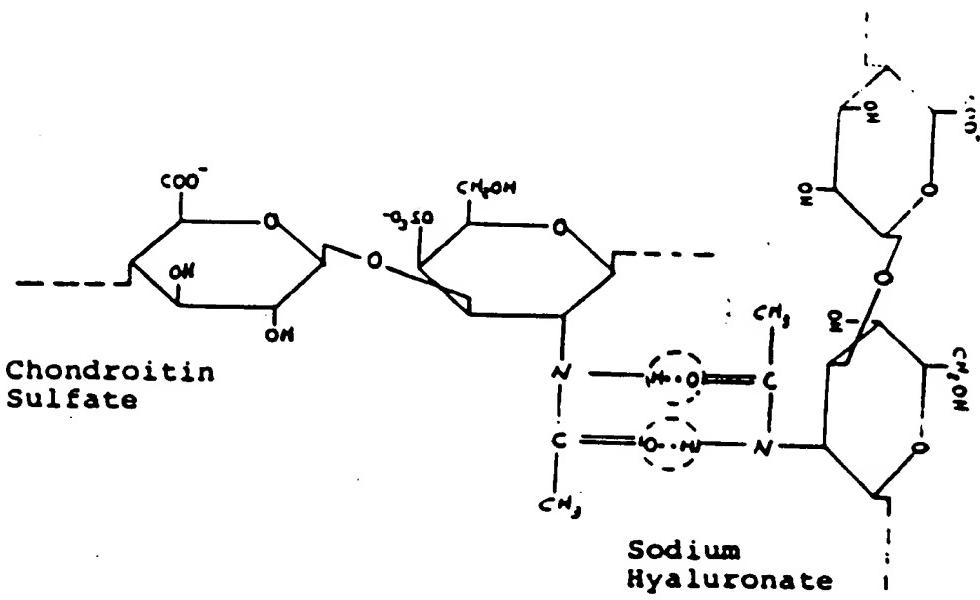
According to another aspect of the invention, there is provided a process of preparing a viscous aqueous composition comprising a chondroitin sulfate and a metal hyaluronate, useful for protecting both human and animal cell layers and tissues subject to exposure to trauma, which comprises mixing the chondroitin sulfate and metal hyaluronate in aqueous buffer solution, in appropriate relevant proportions to cause a significant increase in viscosity of the mixture.

According to yet another aspect of the invention, there is provided a viscous, aqueous composition of chondroitin sulfate and a metal hyaluronate.

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Both chondroitin sulfate and sodium hyaluronate are glycosaminoglycans, commonly known as mucopolysaccharides. By mixing chondroitin sulfate and sodium hyaluronate in aqueous solution, it has been surprisingly found that the molecules appear to line up and attract each other by hydrogen bonding in the N-acetyl amino group as shown below for a segment of molecular units. The hydrogen bonding interaction is only one of several possible interactions for chondroitin sulfate and sodium hyaluronate.



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Turley and Roth, Nature, 283, pp. 268-271 (1980), have experimentally demonstrated that chondroitin sulfate-derivatized beads and hyaluronate-derivatized beads are capable of binding interaction with each other

5 and have postulated that the interaction occurs between the carbohydrate chains of the polymers.

It has been surprisingly found that addition of chondroitin sulfate to sodium hyaluronate in aqueous
10 solution or sodium hyaluronate to chondroitin sulfate in aqueous solution dramatically increases the viscosity of mixture. This increase in viscosity appears to be mainly due to increase in molecular weight rather than solute concentration increase. As those in the art are
15 aware, viscosity of solute-solvent is a function of molecular weight and concentration of solute. The hydrogen bonding interaction postulated above between chondroitin sulfate and sodium hyaluronate would result in effectively enlarging the molecular size. Thus,
20 viscosity of the mixture would be increased. The following examples demonstrate the nonlinear or synergistic change in physical properties by mixing of chondroitin sulfate and sodium hyaluronate in aqueous solution, which characterize the present invention. In the following
25 examples, chondroitin sulfate is abbreviated CS, and sodium hyaluronate is abbreviated SH.

Examples

A chondroitin sulfate/sodium hyaluronate solution was prepared by adding 5.3 grams of chondroitin sulfate to 4.2 grams of sodium hyaluronate in 100 ml of water containing 0.15 grams of monobasic sodium phosphate and 0.45 grams of ditasic sodium phosphate

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with a trace of sodium chloride. The viscosities of the mixture and individual solutions are shown below.

5

Solution A

5.3 g CS/4.2 SH in 100 ml water with buffer.
Viscosity at 25°C = 71,500 CPS at shear rate less than 2/sec.

10

Solution B

4.2 g SH in 100 ml water with buffer.
15 Viscosity at 25°C = 58,700 CPS at shear rate less than 2/sec.

20

Solution C

5.3 g CS in 100 ml water with buffer.
Viscosity at 25°C = 10 CPS.

25

A negative reaction (i.e., lack of synergistic change) of chondroitin sulfate with methyl cellulose (MC) is demonstrated by the following example. Two grams of chondroitin sulfate is added to 2 grams of methyl cellulose in 100 ml of water.

30

The viscosities of mixture and individual solutions are shown below.

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Solution D

- 2 g MC/2 g CS* in 100 ml water.
Viscosity at 25°C = 5,991 CPS at shear rate less
5 than 50 sec.

*CS is insoluble in solution containing
over 2 g MC.

10

Solution E

- 2 g MC in 100 ml water.
Viscosity at 25°C = 5,857 CPS at shear rate less
than 50 sec.

15

Solution F

- 2 g CS in 100 ml water.
Viscosity at 25°C = 3.0 CPS.

20

- Due to the molecular structure of methyl cellulose, the -N-C- groups in chondroitin sulfate could not possibly form hydrogen bonds with methyl cellulose.
25 Methyl cellulose lacks -N-C- groups in the molecules. Hence, addition of chondroitin sulfate in methyl cellulose solution results in insignificant increase in viscosity. The observed small increase in viscosity is due to concentration effect only.

- 30
- Interaction between chondroitin sulfate and sodium hyaluronate is believed to take place at any concentration. However, the synergistic viscosity effect increases with increasing concentration because of concentration effect and closeness of

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molecules for interaction. The solution of chondroitin sulfate/sodium hyaluronate mixture exhibits non-newtonian flow characteristics and has pseudo-plastic behavior. The viscosity of pseudo-plastic substances decrease with increasing shear rates, i.e., relation of viscosity to shear rate is not linear. The following table shows the viscosity plotted against high shear rate at 25°C for solution "A".

10

SHEAR RATES (sec⁻¹)

	<u>250</u>	<u>500</u>	<u>2500</u>	<u>5000</u>
Absolute Viscosity, CPS at 25°C	1,774	1,075	307	181
15				

Most recent data indicate that CS/SH compositions of the invention do not have a yield point.

In the practice of the invention sodium hyaluronate may be used at concentrations from about 0.1 g up to about 10 g in 100 ml water at temperatures from about 4°C to about 37°C. Chondroitin sulfate is also used at concentrations from about 0.1 g up to about 10 g in 100 ml water at temperatures from about 4°C to about 37°C. Within the ranges just described, any quantity of chondroitin sulfate can be added to form binding interaction with hyaluronate and produce physical and flow properties suitable for specific pharmaceutical and ophthalmic uses. Adding 12.6 g of chondroitin sulfate to 10 g sodium hyaluronate in water, the resulting solution has viscosity of over 1 million centipoises at 25°C (for low shear rate below 50 sec⁻¹).

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The aqueous buffer solution used in the practice of the invention includes monobasic sodium phosphate, dibasic sodium phosphate, and sodium chloride mixed to form an aqueous buffer to maintain pH of about 7 to about 8.0 and osmolarity of 300 - 350 mOsmol/kg. By raising the buffer concentration of monobasic sodium phosphate and dibasic sodium phosphate, the ionic strength of chondroitin sulfate/hyaluronate solution is increased. The kinetic rate constant of molecule interaction between chondroitin sulfate and hyaluronate is increased by raising ionic strength and temperature. This invention comprises concentrations of dibasic sodium phosphate and monobasic sodium phosphate from 0.1 g/100 ml to 5 g/100 ml and pH range of 7.0 to 8.0 at reaction temperatures between 4°C and 40°C. The following example shows the effect of buffer on the viscosity or molecular weight of complex molecules for 5.3 g CS/4.2 g SH in 100 ml water:

20 Buffer 1: Dibasic sodium phosphate - 4.5 mg/ml
Sodium dihydrogen phosphate hydrate -
1.5 mg/ml
Viscosity of compositions of the
present invention at 1 second and
25°C is 68,878 cps.

25 Buffer 2: Dibasic sodium phosphate - 7.5 mg/ml
Sodium dihydrogen phosphate hydrate -
1.0 mg/ml
Viscosity of compositions of the
present invention at 1 second and
25°C is 115,011 cps.

30 The solution of chondroitin sulfate/hyaluronate mixture not only exhibits viscoelastic but also rheoplectic behavior (i.e., viscosity increases

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with time at constant shear rate). At constant shear rate of 100 sec⁻¹ for solution A, shear stress increases from 435 pascals to 452 pascals in 3 minutes. Both chondroitin sulfate and hyaluronate are helical straight chain molecules. In highly viscous environments the lower the temperature the less mobility both molecules will have to align the N-acetylamine groups for mutual interaction. This interaction may take place at a very slow rate. However, the mutual interaction increases with raising of the temperature due to kinetic rate increase and better mobility of molecules to align the N-acetylamine groups for hydrogen bonding. When shear energy is added to the molecules, the kinetic energy of the molecules increases and the molecules are more easily aligned or oriented for bonding interaction. Thus, the viscosity is increased when shear energy is added to the mixture and/or the mixture is subjected to an increase in temperature, resulting in high molecular weight material.

Although the invention has been described in detail with reference to chondroitin sulfate and sodium hyaluronate, it is also applicable to sodium, potassium, magnesium and calcium chondroitin sulfates, and to potassium, magnesium and calcium hyaluronates.

It is understood that various other modifications will be apparent to and can readily be made by those skilled in the art without departing from the scope and spirit of this invention. Accordingly, it is not intended that the scope of the claims appended hereto be limited to the description as set forth herein, but rather that the claims be construed as encompassing all the features of patentable novelty which reside in the present invention, including all features which would be treated as equivalents thereof by those skilled in the art to which this invention pertains.

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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A process of preparing a viscous aqueous composition comprising a chondroitin sulfate and a metal hyaluronate, useful for protecting both human and animal cell layers and tissues subject to exposure to trauma, which comprises mixing the chondroitin sulfate and metal hyaluronate in aqueous buffer solution, in appropriate relevant proportions to cause a significant increase in viscosity of the mixture.
2. The process of claim 1, wherein the metal hyaluronate is employed at concentrations from about 0.1 g to about 10 g per 100 ml water, and the chondroitin sulfate is employed at concentrations from about 0.1 to about 10 g per 100 ml water.
3. The process of claim 1, wherein the metal hyaluronate is selected from sodium hyaluronate, magnesium hyaluronate, calcium hyaluronate and potassium hyaluronate, and the chondroitin sulfate is selected from sodium chondroitin sulfate, potassium chondroitin sulfate, calcium chondroitin sulfate and magnesium chondroitin sulfate.
4. A viscous, aqueous composition of chondroitin sulfate and a metal hyaluronate.
5. A viscous, aqueous composition of a metal hyaluronate, at a concentration from about 0.1 g to about 10 g per 100 ml water, and chondroitin sulfate, at a concentration from about 0.1 g per 100 ml water.
6. A viscous aqueous composition of at least one metal hyaluronate selected from sodium hyaluronate, magnesium hyaluronate, calcium hyaluronate and potassium hyaluronate, and at least one chondroitin sulfate selected from sodium chondroitin sulfate, potassium chondroitin sulfate, calcium chondroitin sulfate and magnesium chondroitin sulfate.

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P.E. <i>A 11/23/85</i>	NOV 19 1985	REC'D DEC 15 1987
REPORTS AND ASSESSMENTS - REPORTS & APPENDIXES		

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JULY 2 1970

**Chondroitin Sulfate/Sodium Hyaluronate
Compositions**

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Brown & Orange
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Toronto-Dominion Centre
Toronto, Ontario
MSK 1E6

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B. Aug 18/85											
NOTICE OF ALLOWANCE MAILED AND DISACCEPATION CORRECTED											
DEC 15 1987 6											

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Chondroitin Sulfate/Sodium Hyaluronate

Compositions

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CCA-1954 (part 1) (01-84)

55010-006
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ALLOWED - ACCEPTED
DEC - 2 1987
Ray L. Karsch
SIGNATURE OR EQUVALENT - SIGNATURE D'ÉGALÉTÉ
WITHDRAWN FROM ALLOWANCE - RETIRÉE DE L'ACCÉPATION
RESTORED OR RESTATED - RESTAURÉE OU RÉTABLIE

SECOND ALLOWANCE - REACCETATION

SECOND REFERENCE PATENT - PREMIER DE REVENU

CLAS - CLASSE

REVENUE - REVENU

CLAIMS

REVERGENCE

SECTION NO.

CLAS - CLASSE

REVENUE - REVENU

REVERGENCE

REVENUE

Promulgated, etc.,
Demanding original art, _____
Date, _____

Disclosure	(SIC)	
Dividends	8	comparable
Dissolution	8	comparable
Dividend	8	comparable
Claims	8	comparable
Partnership	8	comparable

Journal of
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FIELD OF SEARCH
CHAMP DE RECHERCHE

DWGS. CORN DESESSN'S	TOTAL
	CURRENT BALANCE
	BALANCE SOLDS
	REMARKS REMARKS
	DETAILS DETAILS

CROSS REFERENCE
RENOVATION

**CORRESPONDING FOREIGN PATENTS
BREVETS STRANGERS CORRESPONDANTS**

Notes